Study on Acaricidal Activities of Selected Phytoextracts against Common cattle tick, *Rhipicephalus microplus*

SYNOPSIS

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INTRODUCTION

Ticks (Phylum: Arthropoda; Class: Arachnida; Order: Ixodida) are the most important vector of diseases of many domestic animals, widely distributed in tropical and subtropical countries including India. **Food and Agriculture Organization (FAO, 2004)** has categorised ticks under two Families: (a) Ixodidae (hard ticks) and (b) Argasidae (soft ticks). The economic loss due to tick's infestation has been estimated as 14000-18000 million USD annually and in India the annual loss is 498.7 million USD mainly due to yield loss of milk (8-9ml) with every bite of the female tick **(Minijauw and Mccleod 2003; Soulsby, 2006)**. Therefore, vector-borne diseases, directly or indirectly, affect the growth of live-stock industry. In order to control ticks, chemical acaricides are commonly used throughout the world **(Freitag and Kells 2013; Kishore *et al.* 2017; 2018).** Synthetic acaricides undoubtedly play an important role in control of ticks but they also have exhibited development of resistance, environmental pollution and residues in meat, milk, hide, skin and natural toxicity **(Bhattacharya *et al.* 2003; Graf *et al.* 2004)**.

These issues necessitate the promotion of alternate Tick Control Strategies. Plants have been traditionally used worldwide to control ticks and other Arthropod vectors **(Nawaz *et al.* 2015).** The extracts of plants are mixture of different bioactive compounds, which act more effectively as compared to chemical acaricides with less chance to develop resistance (**Chungsmarnyart *et al.*, 1991**). The botanical derivatives have their distinguished mode of action such as growth inhibition, ovicidal, larvicidal, adulticidal and repellent activities (**Ghosh *et al.* 2007; Kishore *et al.* 2016; Kishore, 2016**). Amongst the different components of integrated vector management system, herbal acaricides have advantages over synthetic acaricides since they are eco-friendly, cost effective and with minimum environmental toxicity (**Ghosh and Nagar, 2014; Nair *et al.* 2017).**

The impact of botanical derivatives on the morphological, biochemical and physiological parameters of Arthropod vectors is obviously very important. Several workers have reported that phytoextracts can change the morphology, biochemistry and physiology of Arthropods **(Furquim *et al.* 2010; Sharma *et al.* 2011)**.

Multitick infestation is common phenomenon in India, however, amongst the 109 tick species reported from India, *Rhipicephalus microplus* has been considered as one of the most widely distributed tick species **(Ghosh *et al.* 2007)**. Therefore, it necessitates further investigation on the changes and mode of action of phytoextracts on morphological, histological and biochemical changes in the treated-target species. The alterations in the biochemical parameters (lipid, protein and carbohydrates) directly affect the lipid metabolism, peroxidation, physiological stress and hormones regulating protein synthesis **(Sharma *et al.* 2011)**. Further, the histochemical study is an another valuable parameter to detect changes such as present or absence, frequency, distribution of proteins, polysaccharides, lipids and certain enzymes in the female ticks (**De Oliveira *et al.* 2005).**

Keeping in view of the above, present study has been planned to determine the acaricidal activities of garlic bulb (*Allium sativum*), papaya seeds (*Carica papaya*) and sweet lime peels of (*Citrus limetta*) against the common cattle tick, *Rhipicephalus microplus*. The success of the work would be helpful in development of ecofriendly and sustainable management of *R. microplus* to protect domestic animals.

REVIEW OF LITERATURE

To understand the previous work done by different researchers, we have studied the relevant available literature, so that we could analyse the work that has been done, identify the gaps in the information and checkout the direction of further work.

Phytoextracts and acaricidal activities:

Chungsamarnyart and Jansawan (1996) studied the acaricidal activity of peel oil of *Citrus* spp. on *Boophilus microplus*. **Gardulf *et al.* (2004)** showed the protective effect of lemon and eucalyptus extracts against tick and found that it can be an alternative for reducing the number of tick bites, which limits the tick-borne infection. **Abdel-Shafy *et al.* (2006)** evaluated the six wild plant crude extracts of *Artemisia herba-alba, Artemisia monosperma, Euphorbia aegyptiaca, Francoeuria crispa, Mesembryanthemus forsskale* and *Reaemuria hirtela* and screened their acaricidal activity against the larvae of *Hyalomma dromedarii*. **Vatsya *et al.* (2006)** studied the *in vitro* acaricidal effect of hydrodistilled extracts of three medicinal plants *Artemisia annua, A. vulgaris* and *Ocimum kilimandscharicum* and oil seeds of *Pongamia glabra*; out of these, the extract of *Ocimum kilimandscharicum* showed the maximum efficacy against *Boophilus microplus*. **Kumar *et al.* (2008)** screened the effect of four plant extracts: *Nicotiana tobacum, Annona squmossa, Nerium oleander* and *Datura stramonium* on the eggs hatchability of *Boophilus microplus.* **Srivastava *et al.* (2008)** evaluated the efficacy of extracts of leaf, bark and seeds of *Azadirachta indica*, seeds of *Prunus persica*, bark of *Mangnifera indica* and leaf of *Psidium guajava* against *Boophilus microplus.* **Abo-Moch *et al.* (2010)** evaluated the significant acaricidal activity of pomegranate peel and heartwood extract against carmine spider mite, *Tetranychus cinnabarinus*. **Kamaraj *et al.* (2010)** studied the antiparasitic activities of leaf, flower and seed extracts of *Cassia auriculata, Rhinacanthus nasutus, Solanum torvum, Terminalia chebula* and *Vitex negundo* against larvae of cattle tick *Rhipicephalus* (*Boophilus*) *microplus,* adult of *Haemaphysalis bispinosa, Hippobosca maculata*, nymph of goat-lice *Damalinia caprae* and adult sheep parasite *Paramphistomum cervi*. **Rosado-Aguilar *et al.* (2010)** studied the acaricidal activity of crude extracts and fractions of stems and leaves of *Petiveria alliance* against the larvae and adults of

the cattle tick, *Rhipicephalus (Boophilus) microplus* using the larval immersion test (LIT) and adult immersion test (AIT), respectively. **Kumar *et al.* (2011)** evaluated the acaricidal activity of some indigenous plants both *in-vivo* and *in*-vitro in natural tick infestation and reported that *in vitro* mortality percentage was higher with the increased concentration of the extract and the time interval progresses. **Fernandes-Salas *et al.* (2011)** evaluated the *in-vitro* acaricidal effects of lyophilized extracts of four tannin-rich plants, namely *Acacia pennatula, Piscidia piscipula, Leucaena leucocephala* and *Lysiloma latisiliquum* against *Rhipicephalus* (*Boophilus*) *microplus.* **Ghosh *et al.* (2011)** studied the *in-vitro* and *in-vivo* efficacy of 34 plant extracts; among these rhizomes of *Acorus calamus* showed highly efficacious activity against *R. (B.) microplus.* **Martinez-Velazques *et al.* (2011)** evaluated the acaricidal activity of essential oils extracted from cumin seeds (*Cuminum cyminum),* allspice berries (*Pimento dioica)* and basil leaves (*Ocimum basilicum)* against *R. (B.) microplus* tick larvae using the larval packet test (LPT). **Ravindran *et al.* (2011)** studied the acaricidal activity of ethanolic extract of *Leucas aspera* against *Rhipicephalus* (*Boophilus*) *annulatus.* **Juliet *et al.* (2012)** studied the effect of ethanolic extract of leaves of the plant, *Jatropha curcus* as a step toward developing a safe and eco-friendly therapeutic agent to combat the problems of tick and tick-borne disease. **Shyama *et al.* (2014)** investigated the acaricidal effect of *Datura stramonium, Azadirachta indica,* and *Calotropis procera* leaves, *Allium sativum* cloves, and *Carica papaya* seeds extract against cattle tick *Rhipicephalus* (*Boophilus*) *microplus* using *in vitro* studies. **Hai and Atsushi, (2014)** investigated the *in-vitro* and *in-vivo* effect of *Camellia sasanqua* thumb seed oil on the cattle tick, *R. (B.) microplus* and the dog tick, *Rhipicephalus sanguineus,* in Vietnam. **Krishna *et al.* (2014)** studied the acaricidal activity of the petroleum ether extract of leaves of *tetrastigma leucostaphylum* (Dennst.) against *Rhipicephalus (Boophilus) annulatus* using adult immersion test (AIT). **Godara *et al.* (2014)** evaluated the *in-vitro* efficacy of extracts of *Artemisia absinthium* in comparison to amitraz on adults, eggs and larvae of dog tick, *R. sanguineus.* **Parte *et al.* (2014)** evaluated the acaricidal activity of aqueous extracts of five plants (*Azadirachta indica, Mangnifera indica, Polyalthia longifolia, Annona squamosa, Ficus benghalensis)* against cattle tick, *R. (B.) microplus*. **Kishore *et al.* (2015)** evaluated the relative toxicity of certain extracts of *Adhatoda vasica* roots against *R.(B.) microplus.* **Nyabayo *et al.* (2015)** evaluated the acute toxicity

of essential oil of *Salvia nolotica* (sage) against *Rhipicephalus appenduculatus.* **Nawaz *et al.* (2015)** evaluated anti-tick activity of plants against 12-14 days old larvae of *R. microplus* by using water extract prepared from leaves of *Azadirachta indica, Dalbergia sisso and Morus alba.* **Nyigo *et al.* (2016)** investigated the acaricidal effect of extracts from *Synadenium glaucescens* (Euphorbiaceae) on *Boophilus decoloratus* and *B. microplus* ticks. **Rosado-Aguilar *et al.* (2017)** studied the acaricidal activity of methanolic extracts from the leaves of *Havardia albicans* and *Caesalpinia gaumeri* were tested on the larvae and adult of the cattle tick *R. microplus.* **Valente *et al.* (2017)** studied the efficacy of biotheratic and 5% eugenol for the control of *Rhipicephalus* in artificially infested calves. **Dantas *et al.* (2017)** investigated the acaricidal activity of leaves of *Morus nigra* against the cattle tick, *R. microplus.* **Bhikane *et al.* (2017)** assessed the acaricidal activity of polyherbal spray of *Andropogon citrates*, *Cymbopogon citratus*, *Ocimum sanctum*, *Pinus longifoia*, *Calotropis procera*, *Datura stramonium*, *Aegle marmelos*, *Ricinus communis*, *Azadirachta indica*, *Allium sativum*, *Carica papaya*, *Annona squamosa* and *Pongamia glabra* against cattle tick infestation. **Venturelle *et al.* (2017)** studied the *in vitro* acaricidal activity of essential oil of *Piper nigrum* and *Citrus limonum* against *R.* (*B.*) *microplus.* **Nair *et al.* (2017)** studied the *in vitro* effects of ethanolic extract and its fractions of *Ageratum conyzoides* against common cattle tick *Rhipicephalus annulatus.* **Avinash (2017)** evaluated the acaricidal activity of leaf extracts of *Azadirachta indica* and reported that the hexane leaf extract was highly potent against the larvae of *Rhipicephalus* (*Boophilus*) *microplus.*

Biochemical and histological studies:

Matsuo *et al.* (2003) studied the extracellular structure of the midgut of a tick *Haemaphysalis longicornis.* **Villarino *et al.* (2003)** investigated the biochemical assay for detection of esterase activity in the tick integument. **Denardi *et al.* (2004)** studied the morphological characterization of the ovary and vitellogenesis dynamics in the tick *Ambylomma cajennense.* **Baffi *et al.* (2005)** compared the esterase profile of susceptible and cypermethrin-resistant strains of adult *B. microplus* and a pyrethroid susceptible reference strain using polyacrylamide gel electrophoresis and specific staining. **De Oliveira *et al.* (2005)** studied the morphology of the ovary and vitellogenesis process in

oocyte of tick *Rhipicephalus sanguineus*. **Denardi *et al.* (2010)** studied the morphological changes in the oocytes of *R. sanguineus* treated with aqueous extract of leaves of *Azadirachta indica*. **Furquim *et al.* (2010)** studied the changes undergone by cells of the salivary glands of unfed and feeding in *R. sanguineus* as well as new cell types. **Roma *et al*. (2011)** evaluated the effects of the permethrin pyrethroid on oocytes of *R. sanguineus* fully engorged females to determine the cytotoxic effects. **Al-Mola and Rahemo (2012)** compared the biochemical parameters in the salivary gland of ixodid tick, in which they found that total protein intensity and carbohydrate intensity in salivary extracts was higher in *H. anatolicum* than *R. sanguineus* and lipid intensity in salivary extracts was higher in *R. sanguineus* than *H. anatolicum*. **De Oliveira *et al.* (2013)** studied the effects of the arthropod growth regulator in the formation of integument and digestive processes of *R. sanguineus* nymphs fed on rabbits treated with different doses of fluazuron, chemical acaricide. **De Oliveira *et al.* (2014)** demonstrated the effects of the arthropod growth regulator fluazuron in the formation of the integument and the digestive processes of *R. sanguineus* nymphs. **Angelo *et al*. (2015)** studied the carbohydrate metabolism of *Rhipicephalus microplus* after infection with *Beauveria bassiana* and *Metarhizium anisopliae*. **De Oliveira *et al.* (2016)** studied the effect of dinotefuran in germ cells and the digestive processes of semi-engorged females of *R. sanguineus* and found that some damage occurred in the generative cells of midgut; size of digestive cells etc.

The review of literature revealed that the most of the researchers have paid the attention towards the evaluation of different biological activities of the phytoextracts. Cloves of *Allium sativum* and seeds of *Carica papaya* show the acaricidal activity against *Rhipicephalus microplus*. Our laboratory screening the extracts of *Citrus limetta* peels exhibit efficient acaricidal property against *R. microplus*. The extracts of *A. sativum, C papaya* and *C. limetta* have not been explored completely against all the life stages of *R. microplus*. This work gives the basic information about the acaricidal efficiency of the extracts. Further, studies are required to explore these plants' efficacy as ovicidal, larvicidal, adulticidal along with the impact of the extracts on biochemical, morphological and histopathological changes to develop an ecofriendly and efficient acaricide for the sustainable management of the common cattle tick, *R. microplus*.

OBJECTIVES

The aim of the present study is to develop an eco-friendly management of the common cattle tick, *Rhipicephalus microplus.*

- To evaluate the acaricidal bioefficacy of certain phytoextracts (*Allium sativum, Carica papaya* and *Citrus limetta*) against the cattle tick, *Rhipicephalus microplus.*
- To identify the most efficient extract of acaricidal nature on the basis of median lethal concentration against the *Rhipicephalus microplus*.
- To study the impact of the most potent phytoextract on the treated target organism using morphohistological and biochemical parameters.

MATERIALS & METHODS

A. Materials:

Target organism:

Rhipicephalus microplus belongs to Phylum: Arthropoda; Class: Arachnida; Order: Ixodida; Family: Ixodidae; Genus: *Rhipicephalus*; Species: *microplus*. This tick was formerly known as *Boophilus microplus*, however *Boophilus* has recently become a Subgenus of the Genus *Rhipicephalus*. *R. microplus* is being found worldwide in subtropical and tropical regions including India. It is a one-host tick and thus all the stages are present on a single animal. The eggs hatch in the outside environment and the larvae crawl up the grass or other plants to find a suitable host. In the summer, *R. microplus* can survive up to 3-4 months without feeding. In winter, it may live without food up to 6 months. Ticks that do not find a suitable host eventually die due to starvation. The female ticks die after ovipositing. Ticks in the Subgenus *Boophilus* have a life cycle that gets completed in 3-4 weeks and this characteristic can result with heavy tick burden on the host **(Wang *et al.* 2007).**

Selection of plants:

1. ***Allum sativum*** **(Garlic; Lehsun):**

Allium sativum, commonly known as Garlic, is a bulbous plant. It grows up to 1.2 m (4 ft) in height and is domestically stored in warm [above 18 °C (64 °F)] and dried to keep it dormant to inhibit sprouting. It is one of the earth's greatest health tonics and indeed has scientifically proven myriad kinds of medicinal properties. Adulticidal and larvicidal activities of *A. sativum* cloves extract against cattle tick, *R. microplus* has been reported by **Shyama *et al.* 2014.**

2. ***Carica papaya*** **(Papaya; Papita):**

The papaya, papaw or pawpaw, is the fruit of the plant *Carica papaya*, the only species in the Genus: *Carica*; Family: *Caricaceae*. It is a native of the tropics of America and is widely cultivated in Asia, including India. The papaya is a large, tree-like plant, with a single stem growing up to 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The leaves are generally

large, 50–70 cm in diameter and deep palmately lobed with seven lobes. The tree is usually unbranched and the flowers appear on the axils of the leaves, maturing into large fruits. The fruit is ripe when it feels soft and its skin has attained amber to orange hue **(Arvind *et al.* 2013).** Methanolic extract of *C. papaya* seeds has been evaluated for its acaricidal property against the common cattle tick, *R. microplus* by **Shyama *et al.* 2011.**

3. *Citrus limetta* (Sweet lime; Mausmi):

It is commonly known as sweet lime and belongs to Family Rutaceae. *Citrus limetta* is one of the most important commercial fruit crops grown in all continents of the world. Fruits are mainly used by juice processing industries while the peels are considered a waste material. Since the juice yield of *Citrus* is less than half of the fruit weight and thus very large amount of peel waste is formed every year **(Manthey and Grohmann, 2001).** Acaricidal activity of peel oil of *Citrus* spp. on *B. microplus* has been studied by **Chungsamarnyart and Jansawan 1996.**

B. Methodology

Rearing of target organism:

The engorged individuals of the target tick would be collected from cattle sheds of different locations of Agra region. The collected ticks would be transported to the laboratory and subject to identification by using standard tick identification keys **(Walker *et al.* 2003).** After proper identification, the fully engorged adults would be rinsed with water to remove the dust and other foreign particles and be placed on filter paper in the petri dishes. These engorged females would be kept inside the tick rearing glass tubes covered with muslin cloth with the help of rubber band. Glass tubes would be kept in a desiccator containing 10% KOH solution and the desiccator would be kept in Biochemical Oxygen Demand (BOD) incubator at 75±5% relative humidity and 27±2°C temperature **(Bailey, 1960).**

Collection of plant materials and preparation of extracts:

The cloves of *Allium sativum,* seeds of *Carica papaya* and peels of *Citrus limetta* would be collected from the vegetable sellers, fruit sellers and juice makers respectively from the local markets of Agra. The collected materials would be washed in running tap water and dried in shade at room

temperature. The dried plant materials would be grinded to make course powder independently and kept inside the air tight glass containers. The powdered plant materials would be filled in the Soxhlet's apparatus and be subjected for extraction independently using petroleum ether, hexane and methanol subsequently for up to 72 hrs or till the solvent in the siphon tube of an extractor becomes colourless for the complete extraction. The extracts would be taken out, filtered and distilled to concentrate to get the crude extract in rotary evaporator. The extracts would be kept in airtight containers in the refrigerator to avoid loss of volatile compounds **(Kumar *et al.* 2011).**

Bioassay:

The extracts of *Allium sativum, Citrus limetta* and *Carica papaya* would be dissolved in ethanol/acetone to prepare stock solutions of desired concentrations. The series of six test concentrations of increasing order of desired strength in triplicates would be prepared by diluting these stock solutions with distilled water independently. The target organism, *Rhipicephalus microplus* would be exposed to the above test concentrations for 2-3min to evaluate the acaricidal bioefficacy of the extracts separately as per the standard protocol **(FAO, 2004).**

Adult immersion test (AIT):

Five engorged female ticks would be transferred in petri dish. Six groups would be made for treatment along with one control in triplicates. The treated group would be immersed during 2-3min in the prepared desired test concentration of phytoextracts independently and the control group would be immersed in distilled water with maximum amount of the ethanol used in the test concentrations. The ticks would be placed in the desiccator and incubated for a period of 15 days at 27 ± 2^{0}C temperature and 75±5% relative humidity. The treated ticks would be observed under the stereomicroscope and the mortality rate and weight of produced eggs in each group would be recorded daily by counting dead ticks. The dead ticks would be used to observe the effects of extracts on them. Dead ticks would be identified by the presence of cuticular darkness, movement and hemorrhagic skin lesions. After 15 days, the number of females lay eggs would be recorded and the

eggs of each group would be weighted by using an analytical scale. After that 50 eggs would be placed in glass vials at the same conditions. During 21 days, the vials would be observed and the hatching rates of the different treatments would be estimated and compared to the controls. The egg laying inhibition and the larval emergence percentage would be determined for all groups **(FAO, 2004).**

Egg immersion test (EIT):

The engorged females of *R. microplus* would be collected from various animal flocks of different locations of Agra. The collected ticks would be washed in water and then be dried in paper towel. The engorged females would be placed in 30ml capacity of flat bottom glass culture tubes covered with muslin cloth with the help of rubber band. Each culture tube would contain single engorged female for oviposition. The culture tubes would be placed in a dessicator having 10% KOH solution. The dessicator would be kept in the BOD incubator at 27 ± 2^0C and 75±5% relative humidity for two weeks until the eggs are being laid. These eggs would be used for the egg immersion test (EIT). 100-150 eggs would be exposed to the series of the desired test concentrations in triplicate along with control in 10ml capacity of flat bottom glass tubes covered with muslin cloth with the help of rubber band. All the glass tubes would be kept in the BOD incubator for a period of three weeks. The observation would be recorded for the hatching of eggs and the number of hatched and unhatched eggs would be counted and the data would be used for calculating the lethal concentration (**FAO, 2004).**

Larval immersion test (LIT):

The laboratory reared larvae of *R. microplus* of 7-14 days old would be used for experiments. Hatching vials with the highest larvae would be selected and placed in the centre of a petri dish that would be subsequently filled with water and soap to prevent the escape of larvae. A series of different desired test concentration of the above extracts would be prepared independently in triplicate along with control. Approximately 100–150 larvae would be exposed to the above

concentration for 2-3 min in 10ml capacity of flat bottom glass tubes covered with muslin cloth with the help of rubber band. The glass tubes containing treated larvae would be placed in the BOD incubator at same temperature and humidity conditions as above for 24 hrs. Mortality data would be observed after 24hrs of exposure. The larvae that would be unable to walk or move, would considered dead **(FAO, 2004).**

Data analysis:

Bioassay experiments showing more than 20% mortality in control would be discarded and repeated. If the control mortality ranges between 5- 20% would be corrected by using Abbott's formula **(Abbott, 1925).**

$$\text{Corrected percentage mortality} = \frac{\text{\% mortality in treatment- \% mortality in control}}{\text{100- \% mortality in control}} \times 100$$

The observed data would be subjected to Probit analysis **(Finney, 1971)** for calculating $LC_{50,}$ LC_{90} and other statistical values for all the experiments expressed according to the future results.

Biochemical Analysis:

Estimation of total carbohydrate:

Carbohydrate will be determined by the method of **Nelson, 1944**. Total protein would be removed from the tissue homogenate and the filtrate containing glucose only as reducing substrate would be heated with alkaline copper reagent and subsequently treated with arsenomolybdate reagent. The blue colour thus developed, would be read at 540nm **(Sharma *et al.* 2011).**

$$\text{Glucose concentration (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volume of test solution taken}} \times 10$$

Estimation of total lipids:

The lipids will be determined by the method of **Bragdon, 1951** Lipids would be separated from non-lipid components by chloroform-methanol solution estimated in the aqueous phase by the

reducing action of fatty acids on a sulphuric acid-dichromate mixture and the resulting green colour would then read at 600nm **(Sharma *et al.* 2011).**

$$\text{Total lipid concentration (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volume of test solution taken}} \times 10$$

Estimation of total proteins:

The proteins in the homogenates of test and control will be first precipitated by 80% ethanol and the total protein concentrations will be determined by the method of **Lowry *et al.* 1951**.

$$\text{Total protein concentration (mg/g)} = \frac{\text{Concentration of test solution}}{\text{Volume of test solution taken}} \times 10$$

Histological Analysis:

The tissue would be fixed in 4% paraformaldehyde. The material would then be dehydrated in ethanol series, embedded in paraffin wax and then blocks would be made with the help of moulds. The material would be sectioned using a microtome and stained with hematoxylene and eosin following routine histological procedures **(Furquim *et al.* 2010).**

Histochemistry

Histochemical tests would be applied in order to detect the presence of the following compounds: proteins (Bromophenol Blue); lipids (Nile Blue); polysaccharides (simultaneous staining with PAS/ Alcian Blue).

Periodic acid-Schiff technique for polysaccharide detection (Junqueira, 1983)

The phytoextract treated engorged females would be fixed with aqueous Bouin. Slides with section would be immersed for 10 min in 0.4% periodic acid, washed with distilled water, and stained with schiff's reagent for 1hrs in the dark. The material would then be washed thrice with sulfur water for 3 min each and rinsed with tap water for 30 min. after dry, slides would be clarified with xylol and mounted in canada balsam.

Bromophenol blue staining for protein detection (Pearse, 1985)

The phytoextract treated engorged females would be fixed with 4% paraformadehyde. All slides would stain with bromophenol blue for 2hrs at room temperature. Afterwards, they would be washed with 0.5% acetic acid for 5 min and tap water for 15 min; slides would then be quickly immersed in tertiary butyl alcohol, allow to dry at room temperature, clarify and mount in canada balsam.

Baker's method for lipid detection (Baker, 1946)

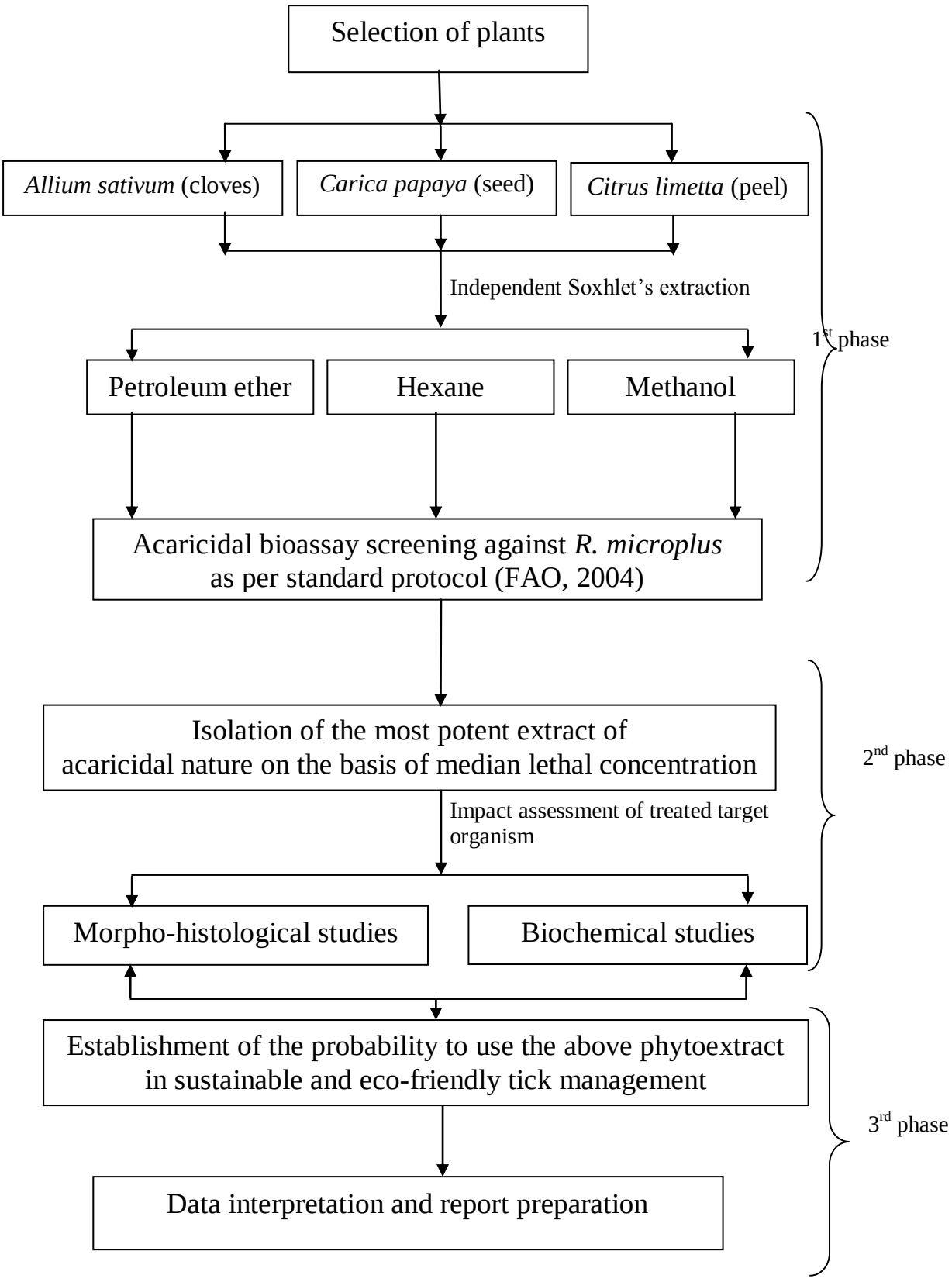
The phytoextract treated engorged females would be fixed with formol calcium for 15hr and transferred to dichromate calcium for 18hr. afterwards, they would be washed with distilled water, and the slides would be immersed in hematein for 5hr. the material would then be rinsed, differentiated in Weigert's solution, and wash with distilled water. After drying, slides would be mounted with glycerine and covered with cover slips.

Morphological Analysis

Scanning Electron Microscopy (SEM).

The collected samples will be fixed in Karnovsky solution and then dehydrated in an ascending series of ethanol, followed by two baths in acetone 100%, also for 15 min each. After critical point drying, the materials will stuck with double-sided tape on aluminum brackets in order to be metalized with gold sputtering. Then, they will be examine and photograph in a SEM **(Remedio *et al.* 2014)**.

METHODOLOGY AT A GLANCE


Selection of plants
Allium sativum (cloves)
Carica papaya (seed)
Citrus limetta (peel)
Independent Soxhlet's extraction
Petroleum ether
Hexane
Methanol
Acaricidal bioassay screening against *R. microplus* as per standard protocol (FAO, 2004)
1st phase
Isolation of the most potent extract of acaricidal nature on the basis of median lethal concentration
Impact assessment of treated target organism
Morpho-histological studies
Biochemical studies
2nd phase
Establishment of the probability to use the above phytoextract in sustainable and eco-friendly tick management
Data interpretation and report preparation
3rd phase

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